BioSystems, 14 (1981) 327—336
Elsevier/North-Holland Scientific Publishers Ltd.

THE MYONEME OF THE ACANTHARIA (PROTOZOA): A NEW MODEL OF CELLULAR MOTILITY*

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(Received June 11th, 1981)

The myonemes of Acantharia are made of bundles of microfilaments twisted up in elementary microstrands of two. Myonemes exhibit three kinds of movements: slow contraction accompanied by undulations of the organelle, quick contraction and subsequent relaxation. In vivo, contraction-relaxation cycles were studied with normal, time-lapse and high speed film-recordings (1000 frames/s). Kinetic parameters of these movements are given. All the movements are actively produced by the organelle itself and are dependant on calcium concentration. An hypothesis about the way myoneme microfilaments might interact to generate motile forces is given. The most closely related system might be the myoneme of the dinoflagellate *Noctiluca miliaris*.

Introduction

Recent cytological and biochemical descriptions of motility processes among unicellular organisms have emphasized the ubiquity of both acto-myosin and tubulin-dynein systems and the evidence that motile forces are generated by the sliding of these microfibrillar proteins (Bloodgood, 1975; Langford and Inoué, 1979; Mooseker and Tilney, 1973; Peltz et al., 1981; Satir, 1968; Satir, 1974; Taylor and Condelis, 1979; Warner, 1976; Woodrum and Linck, 1980). But it now clear that other mechanisms of motility not based on sliding exist in protozoa, as seen in the active bendings of the costa of some trichomonad flagellates (Amos et al., 1979), in the contraction-relaxation cycles of the spasmoneme of the peritrich ciliates (Amos, 1972; Routledge et al., 1976), and in the rowing beats of the axopods of the heliozoan *Sticholonche zanclea* (Cachon et al., 1977).

*This paper is based on an invited presentation made at the Conference on Cellular Evolution (Fourth International Meeting of the Society for Evolutionary Protistology) held 31 May—3 June 1981 at the University of Maryland's Donaldson Brown Conference Center, Port Deposit, Maryland, USA.

Acantharia represent a model of uncommon motility. Compared to the other actinopods, their distinctiveness is mainly due to the presence at the peripheral part of the cell of motile microfibrillar organelles which were previously thought to be cilia (Müller, 1858), then muscles (Haeckel, 1888; Hertwig, 1878). They were called myonemes by Schewiakoff (1926) who observed that their length-change was accompanied by a modification of their structure and that they were sensitive to mechanical or electrical stimulations. Although their structure and behavior recall those of striated muscles, our results suggest a helix-coil mechanism. The following considerations results from the analysis of three kinds of data: time-lapse and high-speed cine-recordings (1000 frames/s), electron microscopy and in vivo and in vitro physiological experiments.

Morphological relationship between myonemes and other cellular structures

Details of the relationship between the myonemes and the other organelles have been previously given (Febvre, 1974). Myonemes are cylindrical or ribbon-like organelles mea-

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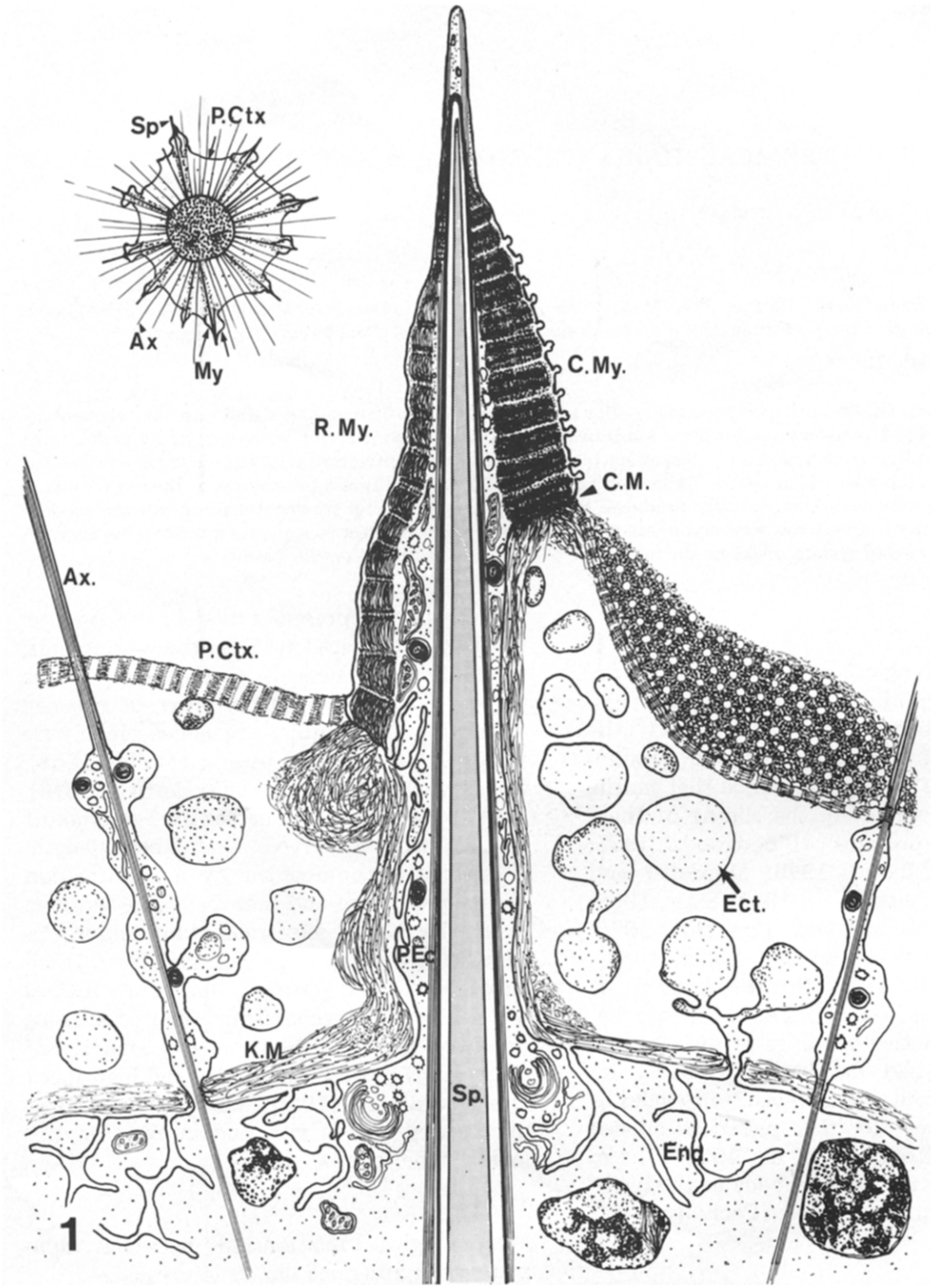


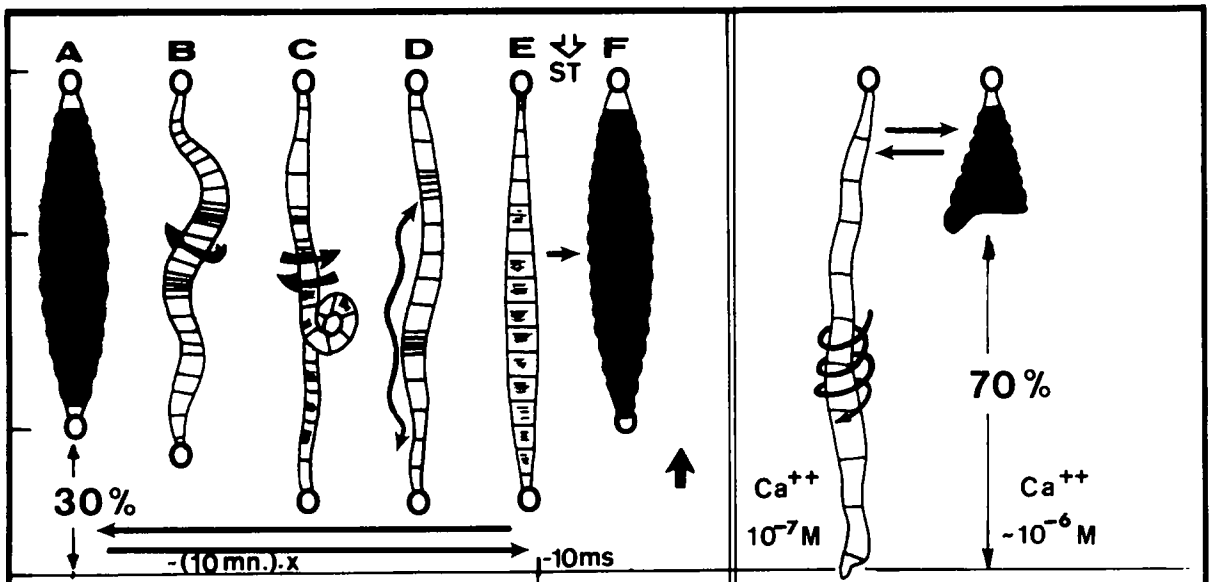
Fig. 1. Interpretative drawing of the perispicular cone at two different phases of the movement. At the left part of the figure, the myoneme is relaxed (R. My), the periplasmic cortex (P. Ctx.) lies close to the ectoplasm (Ect.) and the capsular membrane (K.M.) is thickened. At the right part of the drawing, the myoneme is contracted (C. My.) and the periplasmic cortex is drawn towards the tip of the spicule. The perispicular ectoplasm (P.Ec.) containing some mitochondria and microvesicles lies between the perispicular vacuole surrounding the spicule (Sp.) and the cell membrane (C.M.) Note the outline of the cell membrane at the level of the proximal anchorage of the myoneme. Myoneme microfilaments are inserted on its inner leaflet whereas the cortex is inserted on the outer leaflet.

suring 20—50 μm in length and 0.2 μm in width arranged in rosettes around each spicule (Febvre, 1971) (Fig. 1). They are bound to an outer microfibrillar network called the periplasmic cortex (Febvre, 1972) by their proximal extremity and they are anchored on the membrane of a skeleton-containing vacuole by their distal end (Fig. 7). A diagrammatic representation of the fine structure of the cell in the region of one of the twenty spicules (perispicular cone) illustrates the position of the myonemes in relation to the other organelles of the cell (Fig. 1).

Movements of the myonemes

The motile behavior of the myoneme is complex. The succession of events is never synchronized in the 20 myoneme cones. Moreover one ribbon in one cone usually moves independently of the other ones. Contraction-relaxation cycles of these motor organelles may modify the buoyancy of the protozoan or play a part in expulsion of waste material.

Myonemes exhibit three kinds of movements: slow contraction, accompanied by undulations, rapid contraction and relaxation. In a rosette, only the rapid contraction is synchronous for all the myonemes. During most of the motile cycle, the ribbon waves between its anchor points (Fig. 2B,C,D). A helical component is seen in this movement (Fig. 2B,C, arrows). The cortex remains in



	RELAXATION	CONTRACTION		RELAXATION	CONTRACTION
		ELECTRICAL STIMULATION	MECANICAL STIMULATION		
VELOCITY LENGTH/S	0,02	7	18	0,3	0,3
	IN VIVO			IN VITRO	

Fig. 2. Schematic diagram summarizing the characters of in vivo and in vitro movements of the myonemes.

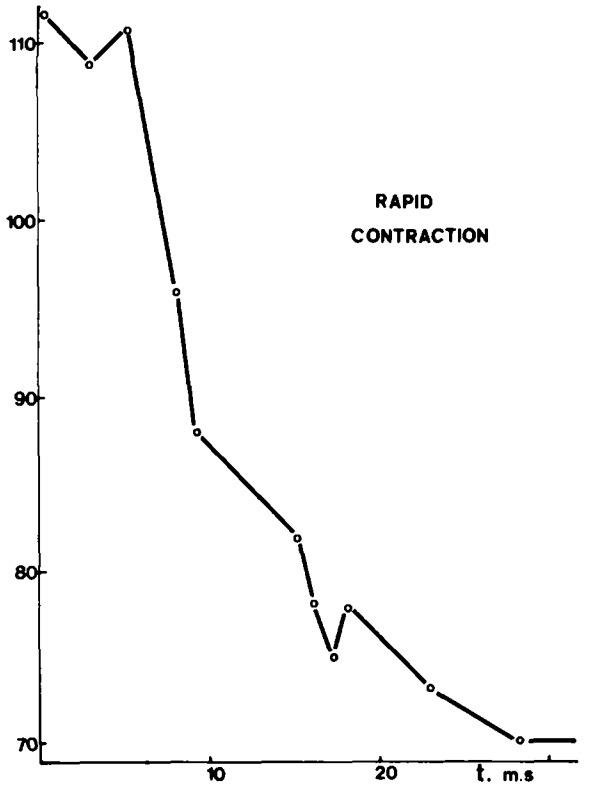


Fig. 3. Kinetic of the rapid contraction. Lengths of the myoneme are given in arbitrary units. The curve is exponential but two small peaks are seen which represent probably the elastic backlash of the cortex.

a low position relative to the tip of the spicule. This phase lasts several minutes. Then, the amplitude of the waves decreases. The myoneme shortens without modifying the position of the cortex. This taut state is transient (Fig. 2E). In calm water, the myoneme relaxes in two or three steps. The slow movement starts again with a smaller amplitude. A series of such cycles results in a stairstep pattern of length changes. The slightest stimulus (Fig. 2, St.), leads all the myonemes of the rosette to contract instantly and rapidly (Fig. 2F).

Recordings at 1000 frames/s makes it possible to analyse the rapid contraction. It lasts about 20 ms and occurs at a propagation velocity of 18 lengths/s. The curve of the length-change is exponential and the extent of contraction is 30% of the maximum length (Fig. 3). The contracted state is brief. Relaxation occurs very slowly at a propagation velocity of 0.02 lengths/s. The magnitude of relaxation is 30% of the extended state and the curve is exponential (Fig. 4). The myoneme relaxes faster than the cortex collapses. Thus the relaxation is active since it is not due

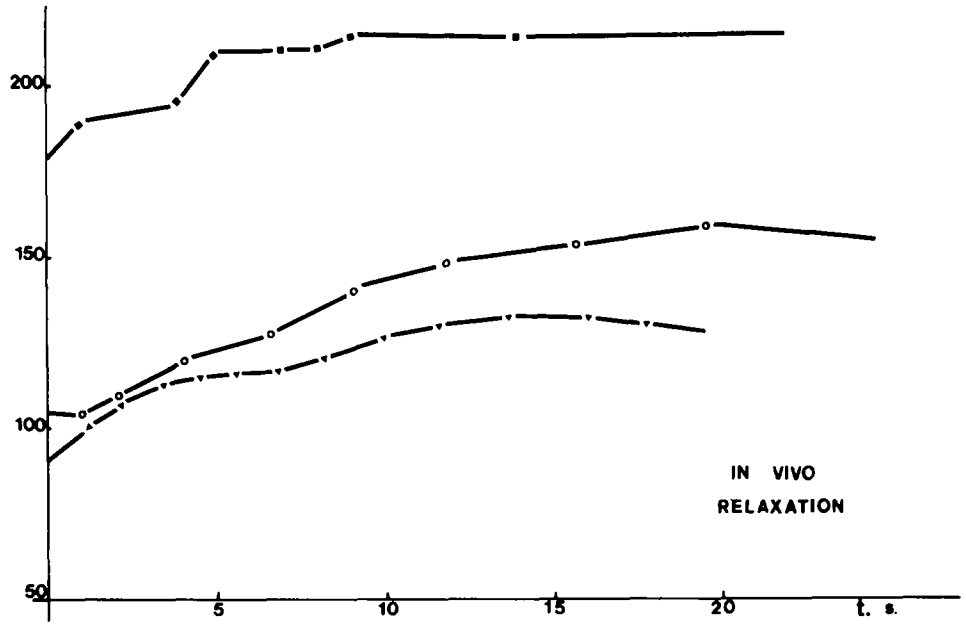


Fig. 4. Kinetic of in vivo relaxation. Three different examples of the exponential curves. Myoneme lengths are plotted in arbitrary units.

to pulling by the cortex. When the myoneme has recovered its maximum length, a new cycle of motility starts again. In a cone, all the myonemes form geometric patterns which are rhythmically reproduced during the slow wave movement (Fig. 5).

When the rapid contraction occurs far from the taut state, one myoneme precedes the other ones and it alone supports the weight of the cortex. The distal anchorage breaks down but the detached myoneme continues to react in phase with other attached myonemes. Its movements are jerky, and the form of the wave is sharp (Fig. 6). The helical component is more obvious than it was previously. The myoneme no longer has the ability to pull the cortex, which demonstrates the active nature of the myoneme. The magnitude of contraction reaches 70% and the curve of the length vs. time is exponential.

Electron microscopical observations

Electron microscopy allows us to investigate the structural basis for these movements.

The organelle is made of a bundle of microfilaments periodically striated by precise transverse bands (TB) of about 30 nm thick. Between them, long clear zones, the L Zones (LZ) are visible (Figs. 7 and 8). High magnifications show that they are made of microfilaments 2–3 nm in diameter twisted up in elementary microstrands of two (Fig. 12), which do not label with heavy meromyosin (HMM) (in a preliminary experiment).

The number and the spacing of the subperiods vary according to the degree of contraction of the myoneme. Some LZ can be partially contracted. In myonemes fixed during the waving phase, a series of contracted LZ can be seen which may correspond to the propagation wave. It occurs from the base to

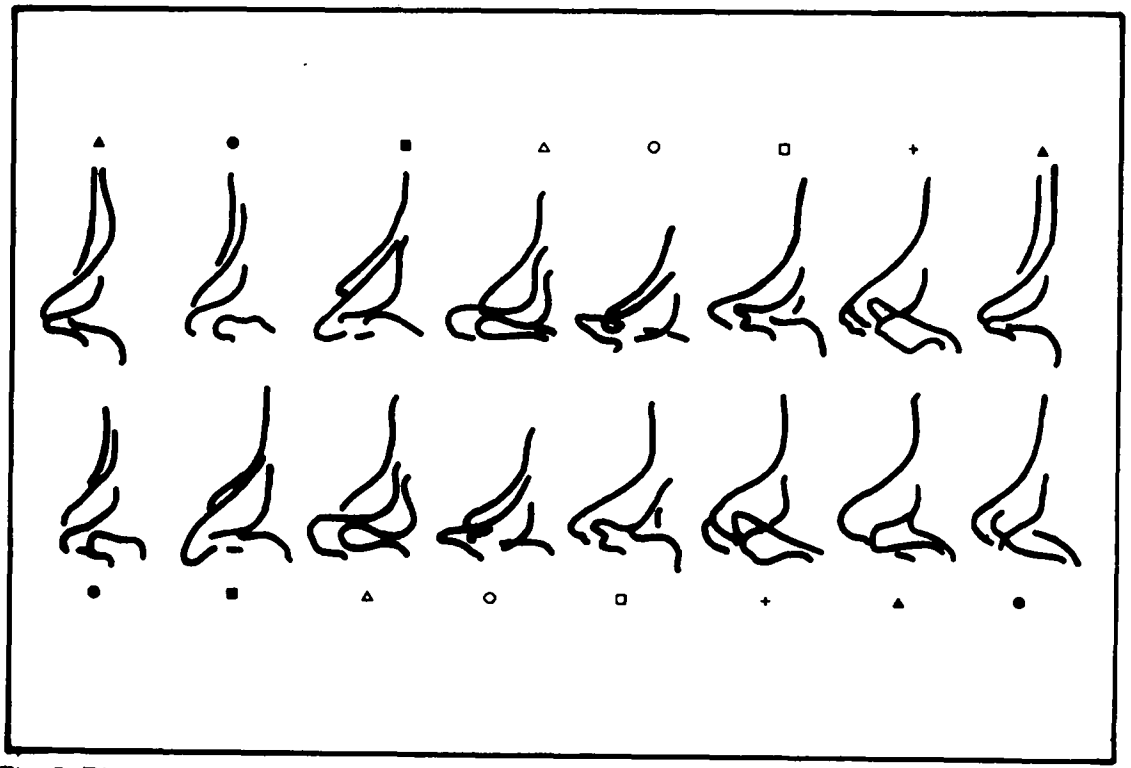


Fig. 5. Diagrammatic representation of the successive positions of the myonemes in one cone from successive frames (5 s between each outline) depicting the rhythmicity of the wave motion.

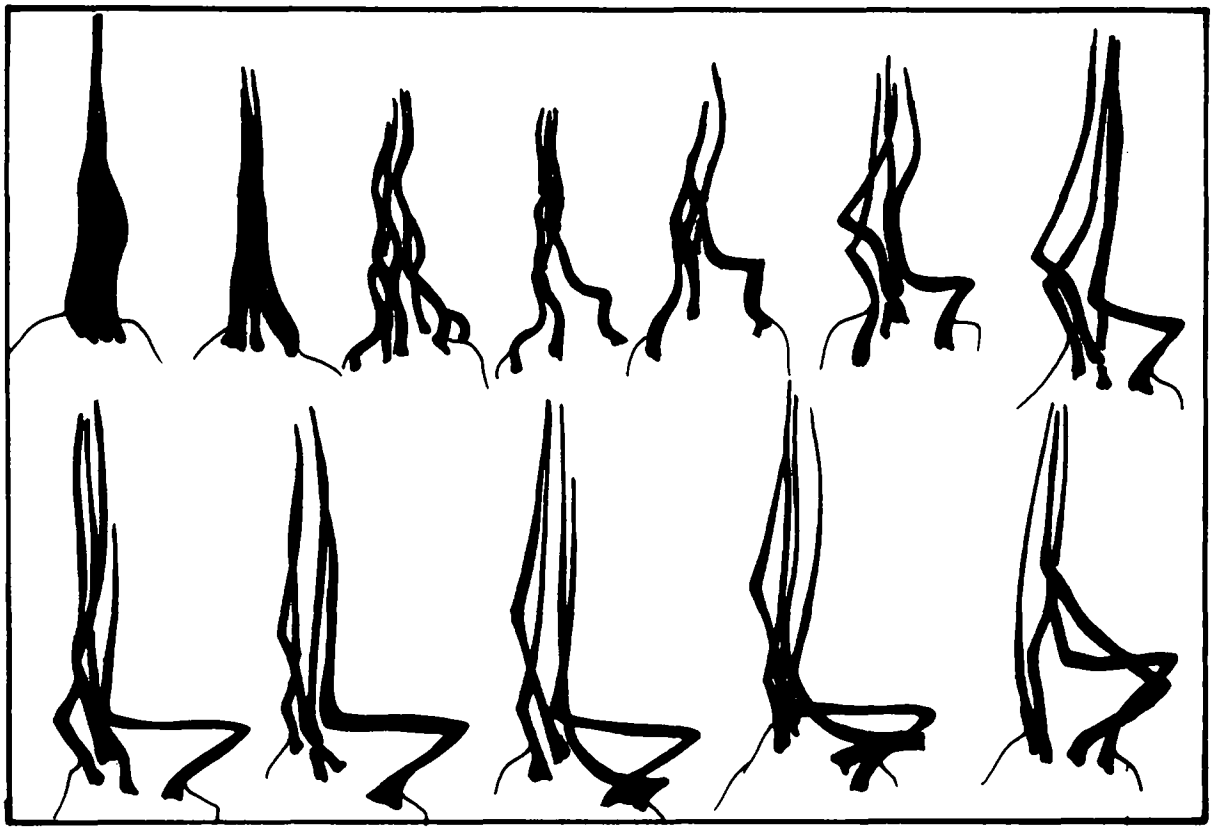


Fig. 6. Wave motion of myonemes which were detached from the apex of the spicule (from successive frames). An acute outline can be seen near to the proximal end of the ribbon.

the tip of the organelle (Fig. 9).

In taut myonemes, all the LZ have the same size, the same number of subperiodic striations and the same appearance (Fig. 7). In myonemes contracted but still attached at both ends, all the LZ are very short with a conspicuous cross periodicity (Fig. 10).

In broken myonemes fixed during the quick

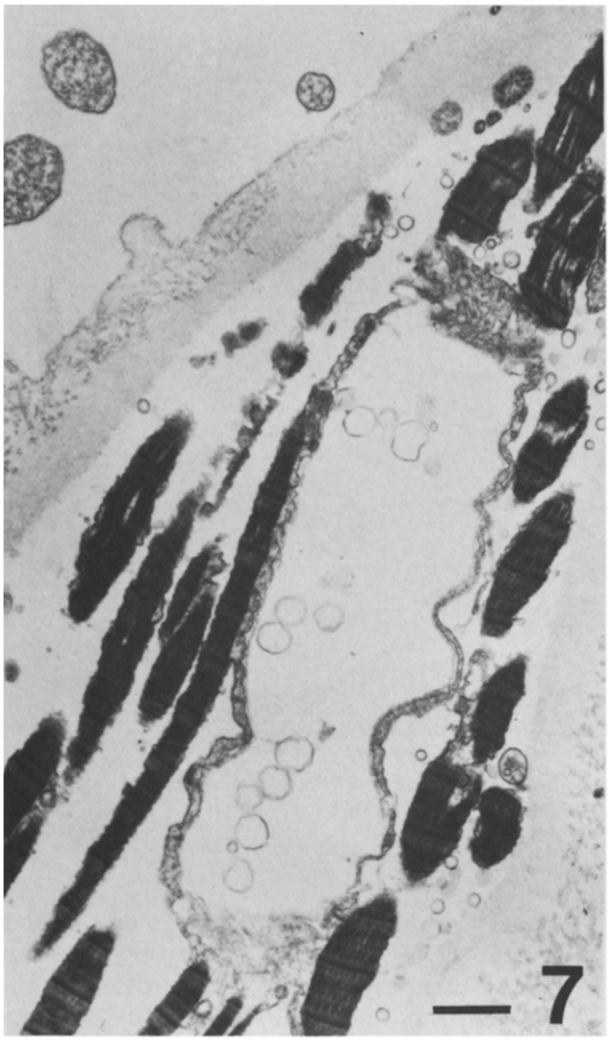
Fig. 7. Survey view of an oblique section through a perispicular cone of *Acantholithium stellatum* showing the trace of the spicule surrounded by the perispicular membrane around which several myonemes lie. The organelle shows dense thin T Bands (TB) regularly spaced and long L Zones (LZ). All of them are equal. Such a feature is characteristic of taut myonemes. The ectoplasmic cortex is visible at the periphery of the perispicular cone. ×10 000.

Fig. 8. Myoneme is made of a dense bundle of microfilaments. It shows periodic striations (TB) alternating with long LZ. Sub-periodic striations can also be seen. In this case, the myoneme is almost totally relaxed, so that these striations are not very conspicuous. ×51 000.

Fig. 9. Myonemes partially contracted, partially relaxed (*A. stellatum*). ×34 000.

Fig. 10. Longitudinal section of contracted myonemes of *A. stellatum* whose anchor points are intact. The apical anchorage is visible (arrow). ×7500.

Fig. 11. Myonemes which have been broken by the fixation. They are supercontracted and the TB which were dark in the extended state appear here less dense. ×34 000.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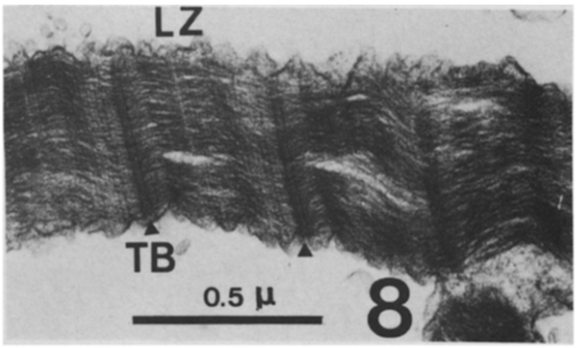
LZ
TB
0.5 μ
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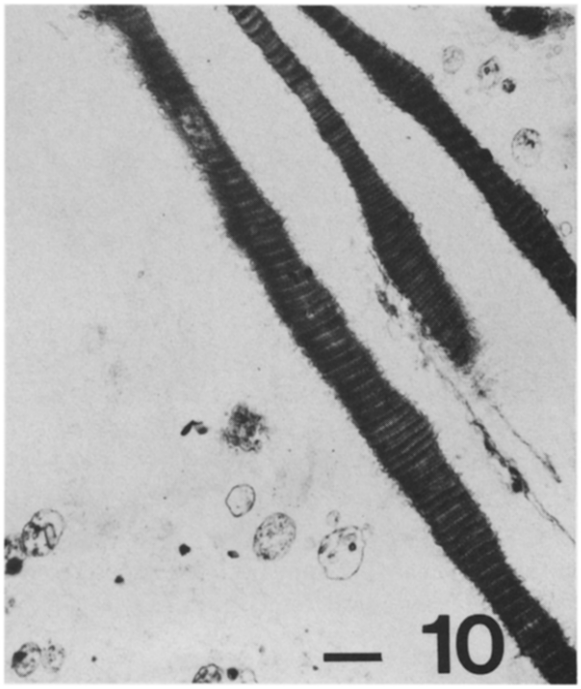
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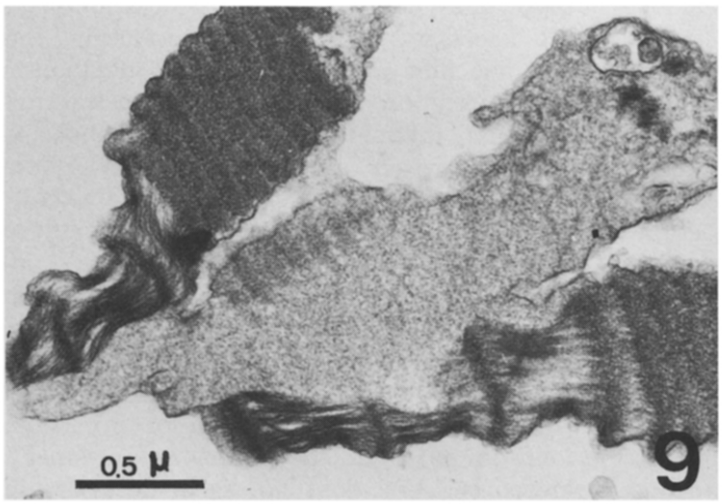
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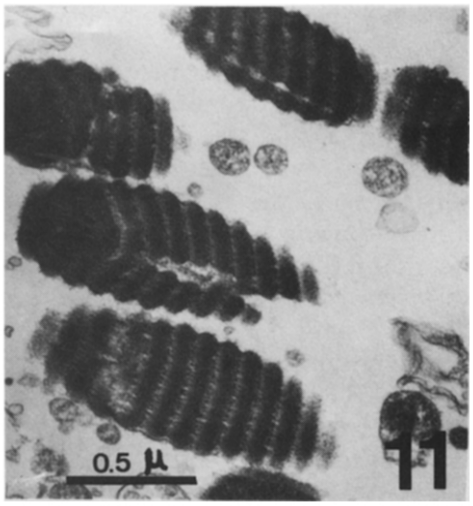
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Fig. 12. High magnification of relaxed myonemes of *Acanthoplegma krohni* showing the relational coiling of the filaments and the microstrands particularly obvious at the level of the TB. × 84 000.

contraction, the LZ appear to be supercoiled and completely compressed (Fig. 11).

Physiological experiments

Physiological experiments are based on action of electric stimulation and on reaction of the myonemes to ionic variations of the medium. In vivo, an electric discharge triggers contraction if the apex of the myoneme is turned towards the cathode. No reaction occurs if it is turned in the opposite direction. Analysis of high speed sequences reveals that this movement is slower (7 length/s) than the rapid contraction carried out in normal conditions (18 lengths/s) (Fig. 2).

The contraction-relaxation movements in vivo are calcium dependent. Below a concentration of 10^{-7} M of calcium, the myoneme extends completely; its contrast vanishes and the helical movement is more obvious than that of intact myonemes. If the concentration of calcium is increased to 5×10^{-6} M, the myonemes contract. Thus the calcium ions are necessary to start the contraction. But does calcium act on the cellular metabolism or directly on myonemes? To answer this question, myonemes have been isolated and demembranated with Triton X-100. Contraction-relaxation cycles are obtained by variation of the concentration of calcium of the medium around the threshold of 0.7 μM. Rapid contraction is never obtained. Both contraction and relaxation velocities are similar (0.3 lengths/s) and the percentage of contraction is 70% of the maximum length (Fig. 2). Magnesium, sodium or potassium ions do not start the myoneme movement. Moreover, ATP does not seem necessary to

induce the movement, but complementary experiments must be performed in order to show the precise role of the nucleotides.

Action of inhibitors of the cellular metabolism like potassium cyanide or sodium azide on live Acantharia stops the myoneme movement. However, the same inhibitor does not act on the movement in vitro and in any case, variations of calcium concentration trigger the movement. These experiments suggest that the myoneme reacts to variations in the intracellular calcium concentration. The fact that slow contraction but not the rapid motion can be started in vitro may indicate that a regulatory protein may bind calcium to particular sites scattered on the microfilaments themselves.

Interpretation of the motility processes

Analysis of the movement, physiological experiments and electron microscopy enables us to suggest a hypothesis about the way by which myoneme microfilaments might interact to generate forces of contraction.

Motility of the myoneme depends on linking and release of sites distributed all along microfibrillar strands. Some of these sites are strongly associated and others are bound in a more unstable manner. They correspond to the sub-periodic striations and induce the movement. Calcium ions trigger the linking of the sites, which progresses from the base to the top of the myoneme as demonstrated by both electric stimulations and electron microscopy. Linkage of the sites triggers a variation of the pitch of the microstrands. During the contraction, the shortening of the microstrands produces the closeness of the TB and the spatial position of the sites is modified, which produces the rotation of the microstrands. The sum of the rotations of all the microstrands induces a torque effect above each TB which modifies the orientation by about one degree for each above LZ. This slight rotation is transmitted to the whole unbound myoneme. Release of sites is induced by the lowering of the calcium concentration. Relaxation is essentially the reverse of the steps involved in contraction.

A detailed study of the myoneme motility will be published in a subsequent paper.

Comparison with other systems of motility

Myonemes may be related to microfilaments bundles of *Sticholonche zanclea*, which are not actin-like and have the same size and show the same relational coiling (Cachon et al., 1977). However, cross striation periodicity varying with the length change is more obvious in the acantharian myonemes than it is in *Sticholonche*.

Myonemes of Acantharia may also be compared to those of the pelagic dinoflagellate *Noctiluca* (Soyer, 1970), *Kofoidinium* and *Pomatodinium* (Cachon and Cachon, this volume) since in both cases the ribbon shaped myonemes are obviously cross striated. But data in these three last genera are still preliminary and preclude a more detailed comparison.

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